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**Angiogenesis in the progression from liver fibrosis to cirrhosis and
hepatocellular carcinoma.**

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Abstract

Introduction: Persistent inflammation and hypoxia are strong stimulus for pathological angiogenesis and vascular remodelling, and are also the most important elements resulting in liver fibrosis. Sustained inflammatory process stimulates fibrosis to the end-point of cirrhosis and sinusoidal portal hypertension is an important feature of cirrhosis. Neovascularization plays a pivotal role in collateral circulation formation of portal vein, mesenteric congestion and high perfusion. Imbalance of hepatic artery and portal vein blood flow leads to the increase of hepatic artery inflow, which is beneficial to the formation of nodules. Angiogenesis contributes to progression from liver fibrosis to cirrhosis and hepatocellular carcinoma (HCC) and anti-angiogenesis therapy can improve liver fibrosis, reduce portal pressure, and prolong overall survival of patients with HCC.

Areas covers: This paper will try to address the difference of the morphological characteristics and mechanisms of neovascularization in the process from liver fibrosis to cirrhosis and HCC and further compare the different efficacy of anti-angiogenesis therapy in these three stages.

Expert opinion: More in-depth understanding of the role of angiogenesis factors and the relationship between angiogenesis and other aspects of the pathogenesis and transformation may be the key to enabling future progress in the treatment of patients with liver fibrosis, cirrhosis and HCC.

Keywords: angiogenesis; liver fibrosis; liver cirrhosis; HCC; progression

Article highlights

- Persistent inflammation and hypoxia are strong stimulus for pathological angiogenesis, vascular remodelling, and liver fibrosis.
- Neovascularization plays a critical role in the progressing from liver fibrosis to cirrhosis and HCC.
- There are different features of angiogenesis in liver fibrosis, cirrhosis and HCC.
- Anti-angiogenesis therapy can improve liver fibrosis, reduce portal pressure, and prolong overall survival of patients with HCC.
- Liver fibrosis, cirrhosis or HCC are multi-factorial diseases, and angiogenesis is only one of factors that favor its genesis and progression. Current approaches to treat liver fibrosis, cirrhosis and HCC using anti-angiogenic therapies are unlikely to be satisfactory.
- TCM has unique advantages in anti-liver fibrosis, cirrhosis and HCC due to its multi-target pharmacological action including anti-angiogenesis efficacy.
- More in-depth understanding of the role of angiogenesis factors and the relationship between angiogenesis and other aspects of the pathogenesis and transformation may be the key to enabling future progress in the treatment of patients with liver fibrosis, cirrhosis and HCC.

1. Introduction

During embryogenesis, vessel formation usually involves two processes including vasculogenesis and angiogenic remodeling. Vasculogenesis refers to the

differentiation and proliferation of endothelial cells (ECs) in situ in the previous non vascular tissues, and then combined to form the original tubular network. Angiogenic remodelling refers to the process in which the primitive network expands to form the interconnecting branching patterns similar to mature vasculature, and the two most important ways are angiogenic sprouting and intussusception [1, 2]. Following this morphogenesis, the normal vasculature becomes largely quiescent. It is generally believed that when the function of pro-angiogenic factors and anti-angiogenic factors is balanced in vivo, the angiogenic switch is off. When the balance is inclined to the direction conducive to angiogenesis, the angiogenic switch is on [3]. In adults, the angiogenic switch is only turned on briefly as part of the physiological processes such as wound healing and female reproductive cycle [4]. Pathological angiogenesis is a hallmark of cancer and various ischemic and inflammatory diseases in which hypoxia and inflammation are strong stimulus for angiogenesis and vascular remodelling [2]. In the mean time, both hypoxia and inflammation are also the most important elements resulting in liver fibrosis, and so angiogenesis is closely related to liver fibrosis and plays an important role in the progression from liver fibrosis to cirrhosis and HCC [5-8].

Previous reviews have described the role of angiogenesis in liver fibrosis, cirrhosis and HCC separately, however, whether there is any different features in angiogenesis during the progression from liver fibrosis to liver cirrhosis and HCC has not been mentioned [5-8]. This paper will try to address the difference of the morphological characteristics and mechanisms of neovascularization in the process from liver fibrosis to cirrhosis and HCC and further compare the different efficacy of anti-angiogenesis therapy in these three stages.

2. ANGIOGENESIS IN LIVER FIBROSIS

Under physiologic conditions, the intrahepatic vascular system is composed of different kinds of vessels, such as liver sinusoids, portal venules, hepatic arterioles, central venules, lymphatic vessels, and hepatic stellate cells (HSCs) act as liver specific pericytes [7]. Liver's blood vessels cover either by continuous endothelial cells or fenestrated sinusoidal endothelial cells (SECs), which have the ability to produce the unique angiogenic factors of the liver including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), Kruppel-like factor 2 (KLF2) , Nitric oxide (NO), Endothelial nitric oxide synthase (eNOS), etc. Angiogenesis in physiological liver regeneration is necessary to the formation of new functional sinusoids [9].

Sustained wound healing reaction, which response to the chronic inflammatory, is the reason why liver fibrosis developed [10]. Angiogenesis, a process of forming new blood vessels from pre-existing vessels, is a critical part of wound healing. So unsurprisingly, angiogenesis plays an important role in the development of liver fibrosis [7, 8, 11-13]. Previous research has found the positive correlation between the degree of fibrosis and the activity of angiogenesis [8, 9]. Inflammation and hypoxia that contribute to angiogenesis [14-17] are also commonly observed in the process of liver fibrosis development [18-20]. Persistent inflammation promotes vascular permeability through loosening of inter-endothelial junctions (IEJs). The extravasated plasma proteins and ECM components constitute a scaffold for ECs migration [10]. Macrophages, monocytes, platelets and mast cells are recruited to the inflammatory area under the mediation of chemokines, which leads to the proliferation and migration of vascular endothelial cells and the subsequent generation of new blood vessels. The diameter and length of these new blood vessels are mainly maintained by VEGF, angiopoietin-1 (Ang-1), α_3 and α_5 integrins [10,

16]. With the progression of CLDs, the deposition of ECM components, the formation of fibrous septa, vascular remodeling along with the progressive capillarization of sinuses will lead to the damage of oxygen diffusion and aggravate hypoxia. The degree of hypoxia in liver tissue is closely connected to histopathological changes [14-16]. The presence of hypoxia area in liver parenchyma is also the most obvious stimulation leading to angiogenesis, which can switch on the transcription of angiogenic genes such as VEGF, PDGF through hypoxia inducible factors (HIFs). NO stimulates vasodilatation, whereas VEGF has properties that enhances vascular permeability, induces EC proliferation and leukocyte adhesion, regulate neovessel lumen diameters and organizes vessel network [9]. However, due to the immaturity and permeability of VEGF induced neovascularization, pathological angiogenesis might not be able to correct liver hypoxia. Pathological angiogenesis and hypoxia may cooperate to disrupt normal tissue repair, thus promoting the development of liver fibrosis, and the vicious circle between fibrosis and pathological angiogenesis further aggravates hypoxia [21, 22].

Pericytes need to be recruited for the neovessels to mature, mainly through ECs releasing PDGF. Pericytes release Ang-1, which establishes sufficient connections between ECs and pericytes to stabilize neovascularization [7, 9]. HSCs, which accounts for approximately one-third of non parenchymal cells and 15% of total resident cells in normal human liver, act as liver specific pericytes and play a central role in the progression of liver fibrosis [7, 18]. Thus, HSCs are known as a crossroad between inflammation, fibrosis and angiogenesis [11]. Quiescent HSCs will be transdifferentiated into myofibroblasts, the major driver of liver fibrosis, after being stimulated by a great quantity of growth factors and pro-inflammatory mediators produced by damaged resident liver cells and hypoxia [18]. Proliferating

myofibroblasts are the key source of excess extracellular matrix (ECM) molecules such as collagen type I and III as well as other proteins that constitute pathologic fibrous tissues.

During the initiation and progression of liver fibrosis, activated HSCs express different chemokines including the CC chemokines (CCL2, CCL3, CCL5) and the CXC chemokines (CXCL8, CXCL9, CXCL10, CXCL12) that are also capable of stimulating angiogenesis. CXC chemokines containing the ELR motif (ELR+) stimulate angiogenesis, while chemokines devoid of this motif (ELR-) inhibit it [11, 23-25]. Accumulated evidence suggests HSCs may operate their pro-angiogenic role in a hypoxia-dependent or -independent manner by responding to a number of stimuli. On the one hand, HSCs are sensitive to hypoxia. Once activated, HSCs act as pro-angiogenic cells and may respond to hypoxia in a HIF-1 α related pathway through the increase of VEGF, Ang-1, and their receptors VEGF receptor 2 (VEGFR-2) and Tie-2 [9, 16, 24, 26, 27]. On the other hand, HSCs may operate their pro-angiogenic role in a hypoxia-independent manner by responding to pro-fibrogenic polypeptide mediators like PDGF and leptin. An angiogenic phenotype of HSCs stimulated by PDGF regulates the formation of vascular tube, increases sinusoidal caliber and blood flow. Together with PDGF, HSCs contribute to the modulation of microvascular structure and hepatic microvascular dynamics [11, 27, 28]. Leptin can directly up-regulate VEGF, Ang-1 and monocyte-chemoattractant protein 1 (MCP-1 or CCL2) in human HSCs [29].

Crosstalk between HSCs and other cells of liver, especially liver sinusoidal endothelial cells (LSECs) and microphages, plays a crucial role in angiogenesis. During sustained hepatic injury, LSECs become capillarized and acquire a pro-vasoconstrictive, pro-inflammatory, pro- angiogenic and pro-fibrotic phenotype.

The loss of fenestrae entails disruption of the oxygen supply and appearance of hypoxia leading to accumulation of HIF that stimulate surrounding cells producing angiogenic growth factors including VEGF, PDGF, fibroblast growth factor (FGF), angiopoietins and start new vessel formation. Then LSECs contribute to HSC activation through secreting PDGF, transforming growth factor- β (TGF- β), etc [30]. Activated HSCs recruit macrophages through chemokines such as CCL2 and infiltrating macrophages further activate HSCs. HSCs also promote angiogenesis and portal hypertension (PHT) by producing angiopoietin 1 and angiotensin I and II [30, 31]. Moreover, CCR2⁺ monocyte-derived macrophages are responsible to angiogenesis in liver fibrosis [32].

In addition, there are also a variety of factors participate in angiogenesis. Leukocyte chemokine 2 (LECT2) is a secretory protein mainly produced by hepatocytes and ECs. LECT2/Tie1 signaling involved in liver fibrosis by inhibiting portal vein angiogenesis and inducing LSECs capillarization [33, 34]. Angiopoietin-3 peptide (ANGPTL3) belongs to liver specific angiogenic mediators and induces endothelial cell adhesion and migration by binding with α V β 3 integrin. However, the role of ANGPTL3 in hepatic angiogenesis need to be further confirmed [11, 35,36].

Angiogenesis in liver fibrosis is characterized by intrahepatic vascular remodeling with capillarization of the sinusoids and development of intrahepatic shunts, which lead to increased hepatic resistance and decreased effective hepatocyte perfusion [9, 15, 16]. Formation of neo-vessels is much more prominent under the conditions of bridging or post-necrotic fibrosis than conditions characterized by pericellular or perisinusoidal fibrosis [8, 14, 37, 38]. There is a positive correlation between the intensity of angiogenesis and the inflammatory activity of chronic hepatitis C (CHC). CD34, a marker of neovascularization, is expressed in the lobular

area around the portal vein and increased with the degree of fibrosis. In the case of septal fibrosis, newly formed blood vessels were observed near the fibrous septum [39, 40].

3. Angiogenesis in liver cirrhosis

Liver cirrhosis is the terminal stage of progressive liver fibrosis, which is characterized by PHT, collateral circulation and pseudolobule [41]. The increase of PP to $\geq 6-9$ mmHg indicates mild PHT, while values ≥ 10 mmHg indicates PHT has clinical significance. PHT in cirrhosis is caused by the contraction of hepatic sinuses and the distortion of liver structure caused by the deposition of ECM and the compression of blood vessels by regenerating nodules. PHT leads to the increase of intrahepatic vascular resistance (IHVR), while progressive splanchnic vasodilation increases the flow of portal vein and further aggravates PHT [5,42,43]. Neovascularization plays a pivotal role in liver vascular remodeling, collateral circulation formation of portal vein, mesenteric congestion, inflammation mediated splanchnic vasodilation and high perfusion [44-47].

The process of angiogenesis in CLDs with PHT is complex. There are many mediators involved in angiogenesis and the development of the portosystemic collateral circulation, among which VEGF, VEGFR-2, PDGF, placental growth factor (PlGF), Ang-1 and Ang-2 all play the role of activation or inhibition [8, 22, 48-51].

HSCs up regulate the expression of VEGF, which is a strong angiogenic signal of LSECs. In turn, VEGF autocrine signals from LSECs react on LSECs, forming a vicious cycle, leading to sinusoidal capillary and fiber formation [5, 52]. NAD(P)H oxidase is required for VEGF-induced angiogenesis. In rats with PHT, blockade of

NAD(P)H oxidase significantly undermined splanchnic expression of VEGF, VEGFR-2 and CD31. In the mean time, increased IHVR, decreased portosystemic collateral formation and superior mesenteric arterial flow were observed [53]. In addition to angiogenesis, VEGF is also an effective vasodilator. The upregulation of VEGF signal in mesentery promotes splanchnic vasodilation by increasing local blood flow and mesentery congestion, which is an early key step in the development of hyperdynamic circulation in PHT [8, 52, 54, 55]. Huang et al. confirmed the significant correlation between the expression of VEGF and PP in the viscera of cirrhotic rodents [56]. VEGF-mediated angiogenesis causes formation of portal-systemic collaterals in PHT through VEGFR-2 and the blocking of VEGFR-2 signaling reduced the visceral hyperdynamic circulation, inhibited the formation of portal collateral in rodents without improving PP [48, 50]. However, the inhibition of VEGF by Sorafenib has no significant clinical effect on PP in patients with liver cirrhosis and HCC. Sorafenib may be a non selective inhibition of systemic VEGF signal transduction and other physiological angiogenesis factors, leading to increased risk of endothelial damage and bleeding [57].

PDGF is another important angiogenic growth factor next to VEGF in liver cirrhosis. PDGF exert its cellular effects by binding to α - and β -tyrosine kinase receptors (PDGFR- α and PDGFR- β , respectively). Endothelial cells produce PDGF and stimulate PDGFR- β on HSCs, triggering their activation. PDGF and its receptor PDGFR- β are up-regulated of mesentery in PHT and cirrhosis, and contribute to angiogenesis. By blocking PDGF receptor, PP and collagen content of liver decreased in cirrhotic rats [5, 58, 59]. The combined inhibition of VEGF and PDGF signals in non cirrhotic or hypertensive rats resulted in the decrease of visceral neovascularization, PP, superior mesenteric artery blood flow and portal system

blood supply, suggesting the importance of VEGF/PDGF driven angiogenesis signals in PHT [60]. It should be noted that in the process of neovascularization, VEGF plays a leading role in the initial stage of neovascularization, activating the proliferation of endothelial cells and the subsequent formation of endothelial tubules. However, the maturation of neovascularization is mainly regulated by PDGF, which regulates the combination of endothelial tubules with wall cells and peripheral cells, so as to stabilize the neovascularization architecture [7, 41].

As a homolog of VEGF, PlGF was observed to be upregulated in liver cirrhosis and HCC [45, 61, 62]. Inhibition or blockage of PlGF activity resulted in reduction of angiogenesis, hepatic inflammation, fibrosis, and lower PP in cirrhotic mouse models. Knockout of PlGF gene reduced superior mesenteric blood flow and PP in noncirrhotic and cirrhotic animal models of PHT. Van Steenkiste C et al. detected strong correlation between the increased circulating PlGF levels, the hepatic PlGF protein expression, and the level of liver fibrosis [63]. Interestingly, this correlation has only been found in pathological conditions suggesting that fewer adverse effects may be expected. PlGF antagonists have shown promising effects in preventing angiogenesis, inflammation, PHT and fibrosis in an animal model [45, 63].

Ang-1 and Ang-2 are ligands for tyrosine kinase Tie-2 receptor on endothelial cells that are considered to be important factors in vascular maturation and stability during angiogenesis. Ang-1 is widely expressed in vascular support cells, while Ang-2 expression is limited to sites of vascular remodeling. Ang-2 and Ang-1 have similar binding affinity for Tie-2. Ang-2 antagonizes Ang1-mediated activation of Tie-2, and the final function of Tie-2 signal depends on the quantitative balance between Ang-1 and Ang-2. In normal tissue, Ang-1 appears to work to stabilize blood vessels, and increased Ang-2 expression in areas of remodeling inhibits this

interaction, which destabilizes blood vessel support cells, a step necessary to facilitate vessel proliferation or sprouting in the presence of VEGF [64-67]. After etiological treatment of viral hepatitis, the level of systemic angiopoietin in patients with advanced fibrosis decreased, while the level of plasma Ang-1 in patients with persistent hepatic necrotizing inflammation increased. In addition, up regulation of Ang-2 can predict the occurrence and development of liver cancer in patients with cirrhosis. Blocking Ang-2 signal can reduce inflammation, liver microvascular normalization and liver fibrosis in cirrhotic rats [68-73].

Apelin is an endogenous ligand for Angiotensin II receptor-like 1 (APJ) that is known to influence angiogenesis [74, 75]. Apelin promotes proliferation and migration of vascular endothelial cells and stimulates vascular sprouting in vivo even in the absence of VEGF [76]. PDGF and pro-inflammatory cytokines activate the overexpression of APJ in HSCs, suggesting that APJ may promote vascular remodeling during fibrogenesis [77]. Apelin and APJ have elevated levels in HSCs and hepatocytes of cirrhotic liver in patients and rats [78, 79]. The expression of apelin was weak in LSECs in the early stage of liver cirrhosis and the proliferative capillaries directly connected to the sinuses, but strong in the proliferative capillaries in the late stage of liver cirrhosis [80, 81]. A recent study using apelin inhibitor has shown that in the mesenteric circulation, collateral formation of the portal system is reduced, as well as the expression of angiogenic mediators such as VEGF, PDGF and Ang-2 [82].

The targeted increase of the pigment epithelium-derived factor (PEDF) presents another potential approach for the treatment of PHT, which overexpression of PEDF decreased angiogenesis, ameliorated fibrosis, and reduced PP in BDL rats [83].

Overexpression of endogenous angioinhibitor vasohibin-1 in PHT and cirrhosis

rats can ameliorate pathological angiogenesis, inhibit hepatic stellate cell activation, improve liver fibrosis, and reduce visceral blood flow, portal vein resistance and portal pressure [84].

Serrano CA et al. evaluated the correlation between angiogenic factors in portal circulation and peripheral venous blood with PP and the complications of PHT such as portal-systemic collaterals, in patients with CLDs undergoing liver transplantation [85]. In patients with PHT, a delicate balance in the angiogenic process is achieved by increasing angiogenesis mediators that include pro-angiogenic factors PDGFR- β , Tie-2 and anti-angiogenic factors VEGF-R1 and Ang-2. VEGF levels in peripheral circulating were reduced in patients with portosystemic collaterals and VEGF-R1, Ang-2, and FGF-1 show different concentrations in the portal circulation and peripheral venous blood in patients. There is no correlation with PP for peripheral concentrations, but significantly reduced portal venous levels of Ang-1, PDGF-AB/BB/CC in patients with higher PP [85]. Ang-1 and its natural antagonist Ang-2 played an essential role in normal and pathological angiogenesis as endothelial cell receptor is activated by Ang-1 and inhibited by Ang-2. PDGF also participates in endothelial remodeling [85]. Endothelial dysfunction has been found in patients with CLDs and PHT [86]. There is an inverse correlation of mediators of endothelial dysfunction in portal circulation with the presence of portosystemic collaterals [85].

The process of vascular reconstruction is chaotic during liver cirrhosis. Many newly formed vessels have a "blind end" and only a few can anastomose with the center of portal vein. These vascular structures are irregular, which surrounded by fibrous tissue of containing activated HSCs and myofibroblasts with contractile function. In this condition, increased liver resistance and portal vein pressure will decrease effective perfusion of hepatocytes, aggravate hypoxia, and further stimulate

angiogenesis and fibrogenesis [8, 87-89].

During the liver cirrhosis regression, systemic hemodynamics normalize, however, the increase of splanchnic blood flow and extrahepatic pathological angiogenesis persist [90, 91]. The shunt degree of collateral system decreased, and the vascular structure remained unchanged. In a word, the continuous high visceral inflow and angiogenesis are not able to recover the PHT but subsidize the liver cirrhosis [90, 91]. Despite sustained virological response (SVR) in patients with HCV related cirrhosis, HCC is still possible ($>2\%$ /year) [92-94]. The occurrence of hepatic arterial hypertension could be a factor of hepatocarcinogenesis [91]. In fact, the imbalance of hepatic artery and portal vein blood flow leads to the increase of hepatic artery inflow, which is beneficial to the formation of liver nodules [95, 96].

4. Angiogenesis in hepatocellular carcinoma

Primary liver cancer especially HCC is the sixth most commonly diagnosed cancer [6]. As one of the most aggressive and fast-growing malignant tumors, HCC is the third leading cause of cancer-related death worldwide, after lung and stomach cancer [97-100]. Regardless of the etiology of liver injury, HCC is closely associated with liver fibrosis and cirrhosis. Approximately 80-90% of HCC patients have potential fibrosis, and around one third of cirrhosis patients will develop into HCC [101]. Chronic liver injury, inflammation and liver fibrosis are features of premalignant microenvironment (PME) in HCC [101].

HCC is a well-vascularized tumor in which angiogenesis plays an important part in development, invasion and metastasis [102, 103]. Vascular network creates immunosuppressive environment and helps tumor escape the influence of immune system. Neovascularization is an alternative to cancer cell expansion and tumor

microenvironment maintenance [104]. With increased arterial formation, HCC shows obvious vascular abnormality, tortuous blood vessels, uneven diameter and irregular branches, functional collateral artery formation, hepatic sinusoidal capillary, increased microvessel density [105-108]. There are four forms of angiogenesis and neovascularization in HCC: co-option, angiogenesis (sprouting), angiogenesis (bone marrow-derived endothelial progenitor cells recruitment to increase tumor vascular supply), and intussusception angiogenesis [107]. The morphological characteristics of sprouting angiogenesis are arterialization of its blood supply and sinusoidal capillarization [104, 109]. Arterialization (arteriogenesis) refers to the growth of functional collateral arteries covered by smooth muscle cells in preexisting arteries [104, 107, 109]. Sinusoidal capillarization leads to lumen deformation and loss of EC fenestration and the transformation of fenestrated hepatic sinuses into continuous capillaries [104, 107, 109].

VEGF/VEGFR system is critical in controlling tumor angiogenesis. Circulating VEGF levels increased in HCC and associated with tumor angiogenesis and progression. VEGF increases vascular permeability and promotes the growth, migration and morphogenesis of vascular endothelial cells [110-114]. Overexpression of VEGF results in abnormal vascular leakage in the newly formed vessels that could contribute to interstitial hypertension and severe hypoxia or necrosis, which may eventually lead to malignancy [115, 116].

Some studies argue VEGF is not solely responsible for the angiogenesis of HCC but also a variety of mediators, such as PDGF, FGF, Ang and etc. [117, 118].

PDGF signal is also considered instrumental in the development of HCC. The overexpression of PDGFR- α is related to microvessel density (MVD) and poor prognosis in HCC while the inhibition of PDGF receptor signal can reduce cell

migration in vitro and tumor growth in vivo. By observing the co expression of PDGFR- α , PDGFR- β and VEGF in HCC patients, it is suggested that PDGFR and VEGFR may interact with each other [119, 120].

FGF also contribute to angiogenesis of HCC. Crosstalk of FGF-2 and VEGF-A in the early stage of tumor growth induces tumor neovascularization and further growth, and is related to the increase of capillary sinuses in HCC tumor angiogenesis. FGF stimulates the regulation of integrin expression in the microenvironment, thereby altering the cell parameters required for angiogenesis [103, 121, 122]. The potential synergy between FGF and VEGF pathways may contribute to the resistance of advanced liver cancer to the VEGF inhibitor sorafenib [123, 124]. FGF-19 is a physiological ligand of FGFR4, and FGF19 is overexpressed in about 50% of patients with liver cancer [125-130]. In addition, FGF19 expression is associated with poor prognosis, recurrence and tumor progression in HCC patients [131-133].

The angiopoietin pathway may play a significant role in tumor angiogenesis. Ang-2 levels were observed to be increased in cirrhosis, and even more so in HCC, potentially in coordination with VEGF ligands [103, 134]. The high Ang-2/1 ratio in HCC is closely related to portal vein invasion, tumor diameter and MVD [103, 135]. Serum VEGF and serum Ang-2 levels are independent predictors of survival in patients with advanced HCC. They found that Ang-2 and VEGF levels in serum were predictors of survival, and the median survival of patients with low and high baseline Ang-2 concentrations was 14.1 and 6.3 months, respectively. The median survival of patients with low and high baseline VEGF concentrations was 10.6 and 6.2 months, respectively [103, 136, 137].

Endoglin (CD105) is a transmembrane glycoprotein, one of the co receptors of transforming growth factor beta (TGF- β), and a marker of ECs activation. Endoglin

not only antagonized the inhibitory effect of TGF- β [138], but also controlled the transformation of endothelial progenitor cells to functional epithelial cells [139]. Endoglin preferentially expressed in hemangioendothelial cells of solid tumors (including HCC) [103, 140-142]. The expression of endoglin is related to the stage, differentiation and aggressive behavior of HCC. Endoglin promotes the invasion and metastasis of hepatoma cells by increasing the expression of VEGF [143].

5. Features of angiogenesis in liver fibrosis, cirrhosis and hcc

5.1 Histopathological features

First, during the process of liver fibrosis, persistent inflammation and hypoxia result in hepatic sinusoidal capillarization, intrahepatic shunt, intrahepatic vascular reconstruction. Intrahepatic resistance increased and effective perfusion of hepatocytes decreased, and hypoxia of liver tissue further aggravated [15, 16].

Then, when progression from liver fibrosis to cirrhosis, the vascular structure remains irregular, but with only a few neovascularization can anastomose with the portal vein, some of them have blind ends. The vessels enclosed by the fibrous tissue lead to high liver resistance and PTH. The effective perfusion of hepatocytes further decrease, the hypoxia aggravates, stimulates the generation of blood vessels and fibers, and forms a vicious circle [8, 87-89].

Eventually, progression to HCC is characterized by an increase in arterial formation, a marked abnormality, irregularity and tortuosity of blood vessels, an increase in sinusoidal capillaries, and the formation of functional collateral arteries [105-108].

5.2 Features of angiogenesis

Inflammation and hypoxia are two key elements in angiogenesis during liver fibrosis, cirrhosis and HCC [14-17]. Persistent inflammation leads to LSECs capillarization, impairs oxygen diffusion from the sinusoids to hepatocytes, promotes vascular permeability and enhances the recruitment of macrophages, monocytes, platelets and mast cells mediated by chemokines to inflammatory areas, thus stimulate the proliferation and ECs migration, and then generate new blood vessels [8, 9, 16, 30]. In this condition, the appearance of neovascularization is to solve the hypoxia of liver tissue. However, it is not very clear that angiogenesis is only a homeostatic mechanism to ensure adequate oxygen supply, or an additional pathogenic effect leading to further liver injury [9]. Angiogenesis in liver tissue is characterized by the formation of branches from the existing vascular system. Most of the neo-vessels originate from the small branches of the portal vein, and tend to establish a connection between the portal vein system and the hepatic vein [8, 147, 148].

Allowing ECs to proliferate and migrate is the basic condition for vascular sprouting. Firstly, plasma proteins leak out from the existing vessels and act as temporary scaffolds for EC migration together with ECM. And NO induces vasodilatation and VEGF increases vascular permeability [9, 13]. Secondly, the activities of matrix metalloproteinases (MMPs), urokinase plasminogen activator (uPA) and their corresponding inhibitors, tissue inhibitor of metalloproteinases (TIMPs) and plasminogen activator inhibitor-1 (PAI-1) were increased, which degraded ECM to allow EC migration and diffusion [149]. $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins regulate the detachment/attachment of ECs and ECM, and maintain the communication between EC and its neighbors [150]. Overexpression of growth factors, cytokines and MMPs interferes with physiological angiogenesis and leads to

liver structure and function disorder. Excessive ECM accumulates with the deposition of fibrillar collagen (type I) rather than physiological collagen (type IV), resulting in distortion of sinusoidal structure and loss of specific endothelial fenestration [16]. The deposition of fibrotic tissue causes resistance to blood flow and impairs oxygen delivery to liver parenchyma, further aggravating hypoxia. Hypoxia and capillarized LSECs contribute the activation of HSCs, which produce VEGF, Ang-1, VEGFR-2 and Tie-2 [16]. Activated HSCs are always limited to the edge of tiny and undeveloped septa, rather than larger bridging septa [26]. Paternostro C et al. found that there may be two independent angiogenesis stages in CLDs. The early stage occurs in the developing septum and is regulated by activated HSCs. In the late stage, angiogenic agents were only expressed in endothelial cells and appeared in large and mature fibrous septum where chronic wound healing process is not active, and fibrinogenic transformation is easier to establish [16].

Sustained inflammatory process stimulates fibrosis to the end-point of cirrhosis and sinusoidal PHT is an important feature of liver cirrhosis. Initially, the increase of intrahepatic vascular resistance in CLDs and a variety of pathological events of hepatic sinusoidal circulation, such as structural distortion caused by fibrosis, microvascular thrombosis, dysfunction of LSECs, activation of HSCs, gradually develop PHT [42, 151, 152]. Liver fibrosis is the first factor to explain the increase of resistance in cirrhotic liver. 80% of the increase in portal resistance is due to the structural distortion of the cirrhotic liver, while 20% is due to the reversible and excessive contractile phenotype [153]. Moreover, through perivascular contraction, HSCs are considered to be a key contributor in the dynamic and reversible components of PHT in cirrhosis [154]. NO is an important regulator of normal hepatic vascular tension and PP. The production of NO increases with the increase of

flow, and can also be upregulated by VEGF [154]. LSECs in cirrhosis have a diminished ability to respond to the increase of blood flow, while the production of NO is increased. This disorder leads to the impairment of hepatic microcirculation vasodilation function in patients with liver cirrhosis, which is an important factor leading to hepatic sinusoidal PHT [154]. Pathological angiogenesis in splanchnic circulation is thought to aggravate PHT, because the increase of vascular system caused by angiogenesis can increase the blood flow of portal vein system, thus increasing PP [154]. Besides hepatic vascular system, mesenteric vascular system also plays a key role in PHT. Increased blood flow caused by excessive vasodilation of splanchnic arteries involved in NO overproduction by eNOS. Even with the development of portal collateral circulation, portal pressure still increases due to the increase of visceral flow to the liver [151, 152]. PHT results in splanchnic and systemic arterial vasodilation, leading to the development of a hyperdynamic circulatory syndrome and subsequently to clinically severe complications including gastroesophageal varices and variceal hemorrhage, hepatic encephalopathy from the formation of portosystemic shunts, ascites, and renal failure due to the hepatorenal syndrome [154].

During the liver cirrhosis regression, systemic hemodynamics normalize, however, the increase of splanchnic blood flow and extrahepatic pathological angiogenesis persist [90, 91]. The shunt degree of collateral system decreased, and the vascular structure remained unchanged. The occurrence of hepatic arterial hypertension could be a factor of hepatocarcinogenesis [91]. In fact, the imbalance of hepatic artery and portal vein blood flow leads to the increase of hepatic artery inflow, which is beneficial to the formation of liver nodules [95, 96]. Recently, the concept of premalignant microenvironment (PME) characterized by chronic liver injury,

inflammation and fibrosis has been proposed. HCC is closely related to liver fibrosis and cirrhosis, suggesting that environment in which HCC elevates may affect tumorigenesis [101, 155]. In addition, cancer-associated fibroblasts (CAFs) of tumor Microenvironment in HCC most likely derive from HSCs, and CAFs play an important part in hypoxia-dependent tumor neo-angiogenesis [101, 156].

In summary, persistent inflammation and hypoxia are the main causes of angiogenesis, and neovascularization is initially formed to improve liver tissue hypoxia. However, pathological neovascularization could not alleviate hypoxia, led to the production of a series of inflammatory factors, chemical factors and pro-fibrotic factors, and recruit a variety of immune cells to the inflammatory areas. In this condition, liver fibrosis forms and gradually progresses to cirrhosis or HCC.

6. Anti-angiogenesis therapy

6.1 Tyrosine kinase inhibitors (TKIs)

Receptor tyrosine kinases (RTKs) family includes many receptors of growth factor family, such as VEGF, PDGF, FGF and epidermal growth factor (EGF). When the ligand binds to the receptor, it activates the receptor tyrosine kinase domain and upregulates the downstream signaling system. These kinases are up regulated in liver fibrosis, cirrhosis, and HCC are considered to be attractive therapeutic targets [157-168]. In recent years, several TKIs such as sorafenib and lenvatinib, have been approved for the treatment of HCC, and some other TKIs are still in clinical trials. Increasing evidence has shown that TKIs is also involved in the therapy of liver fibrosis and cirrhosis [167].

Sorafenib is the first anti-angiogenic receptor TKI, targeting VEGFR-1/2/3, PDGFR- β , and c-Kit receptor. Sorafenib prolonged median overall survival (OS) and

the time to progression by nearly 3 months in patients with advanced HCC in SHARP trial. In 2007, Sorafenib was approved by the FDA for the treatment in advanced HCC [169] and is now recognized as a standard treatment for patients with advanced HCC [170-173]. Sorafenib inhibits HIF-1 α protein synthesis induced by hypoxia, lowering VEGF expression in different hepatoma cell lines and xenotransplantation mice. By blocking HIF-1 α /VEGF pathway, Sorafenib reduces MVD and inhibited tumor vascularization [174-176]. Interestingly, during anti-HCC treatment, clinicians observed that sorafenib had positive side effects on liver cirrhosis [177]. A large number of experimental studies demonstrated Sorafenib's anti-fibrotic effect. In almost all animal models of liver fibrosis, including carbontetrachloride, bile duct ligation, dimethylnitrosamine, diethylnitrosamine, or orthioacetamide, sorafenib showed anti-fibrotic effect [170, 178-181]. Moreover, Sorafenib can reduce the wrapping of contractile HSC around LSECs, regulate the connection complex formed between LSECs, and alleviate fibrosis by Inhibiting VEGF, VEGFR-2, PDGF, PDGFR- β , Tie-2, and etc. [182, 183]. Sorafenib can also reduce PP, decrease the hyperdynamic splanchnic circulation, hinder portosystemic collateralization, and attenuate the complications of liver cirrhosis [47, 57, 167, 183-192]. Sorafenib may have synergistic effect when working with propranolol to inhibit angiogenesis [193].

Lenvatinib, an oral multi TKI, targeting VEGFR 1-3, FGFR 1-4, PDGFR α , c-Kit and RET, was first approved for the treatment of refractory thyroid carcinoma [160, 194, 195], and then by FDA for treating HCC in 2018 [196]. The results of a global phase III trial (REFLECT) showed Lenvatinib was non-inferiority to sorafenib in terms of OS and improved progression-free survival (PFS), time to treatment progression (TTP) and objective response rate (ORR) in the treatment of patients with unresectable HCC [160, 196]. Lenvatinib targeted VEGF and FGF-induced

angiogenesis and presented stronger antiangiogenic effects than sorafenib in HCC transplanted tumor models, especially in patient-derived xenograft model [160, 166, 197-202]. There is limited research on lenvatinib's effects on liver fibrosis and cirrhosis.

Regorafenib, another oral multi TKI, is approved to treat metastasis from colorectal cancer and advanced gastrointestinal stromal tumors through influencing angiogenesis, tumorigenesis, metastasis and tumor immunity [203-206]. In a randomized, placebo-controlled, phase III RESORCE trial, Regorafenib proved its effectiveness as a second-line therapy for HCC and in prolonging survival in patients with tumor progression after the administration of Sorafenib in 2016 [166, 207-216]. Though sharing some similarities with Sorafenib, Regorafenib has stronger anti-tumor and anti-angiogenic effects, significantly improving the survival rate of mice with liver cancer [217]. Long term treatment with Regorafenib reduced PHT, most likely due to decreased angiogenesis without affecting the progression or regression of fibrosis [218].

Cabozantinib is an oral TKI that targets multiple receptor tyrosine kinases, including VEGFRs, mesenchymal-epithelial transition factor (MET), AXL, RET, KIT, and FLT3. It was approved by FDA for the treatment of Medullary Thyroid Cancer (MTC) in 2012 [219-223]. Based on the results of the phase III CELESTIAL trial [224], Cabozantinib, as a new second-line and the only third-line treatment for patients with advanced HCC, was later approved by the FDA for the treatment of patients with HCC who have been previously treated with sorafenib [160, 161, 224, 225-227]. Data from CELESTIAL trials clearly demonstrate the survival benefits of a sustained antiangiogenic strategy that includes continuous use of sorafenib and cabozantinib as second-line therapies [225]. Some HCC patients with high level of

MET are resistant to the Sorafenib treatment. The dual blockade of VEGFR-2 and MET by Cabozantinib can suppress tumor growth, metastasis, angiogenesis and has significant antitumor activities in HCC [222, 228]. There are no further studies of Cabozantinib on liver fibrosis and cirrhosis.

Axitinib is an effective and selective VEGFR (1-3) inhibitor that shows certain clinical results in HCC patients pretreated with VEGF [229]. Axitinib combined with best supportive care (BSC) failed to improve OS compared with placebo/BSC. However, Axitinib/BSC resulted in significant longer PFS and TTP and higher clinical benefit rate (CBR) [230]. After the failure of the first-line drug Sorafenib, the second-line Axitinib exhibited moderate effects in patients with advanced HCC [231].

Since the approval of sorafenib, new candidate AKIs, such as Sunitinib [232], Brivanib [233], Linifanib [234] and Tivantinib [235] have failed to demonstrate their efficacy as first-line therapies against Sorafenib. There are ongoing multicenter, randomized, double-blind, phase III trial evaluating the efficacy and safety of new TKIs including Dornafenib or Apatinib in patients with advanced HCC [162].

6.2 Monoclonal antibodies

The binding of VEGF ligands and receptors plays a pivotal role in initiating signal cascade, angiogenesis, endothelial cell proliferation and migration, and increased vascular permeability. Blocking the binding of VEGF ligands and receptors is expected to prevent the formation of new blood vessels, thus limiting the supply of tumor nutrients and eventually, leading to the death of tumor cells. Several monoclonal antibodies against VEGF or VEGFR, including Bevacizumab and Ramucirumab have been approved for tumor treatment.

Bevacizumab is a humanized monoclonal antibody against VEGF and effectively neutralizes VEGF through VEGFR-1 and VEGFR-2 receptors, blocks its signal transduction, and inhibits VEGF induced angiogenesis, cell proliferation, survival, permeability, nitric oxide production, migration and tissue factor production [236-238]. It was first approved by the FDA as a first-line treatment for extensive metastatic colorectal cancer [239]. Bevacizumab might be a suitable agent for liver fibrosis since it alleviates liver fibrosis in vivo by neutralizing VEGF produced by hepatocytes and by blocking HSCs activation [240]. However, for advanced HCC, bevacizumab combined with erlotinib, which is a multitargeted TKI with activity against Raf kinases via the Raf/MAPK (mitogen-activated protein kinase)/ERK (extracellular signal-regulated kinase) pathway, VEGF, PDGF, and c-Kit, is not better than sorafenib [241]. In patients with unresectable HCC, the combination of bevacizumab and atezolizumab, which achieve dual blockade of VEGF and PD-L1, has better OS and PFS than sorafenib or atezolizumab alone. Severe toxic reactions occurred in 38% of the patients receiving the combination therapy, however, no new or unexpected toxic effects were observed [242, 243]. Inhibition of VEGF inducible angiogenesis can reduce tumor hypoxia, transform immunosuppressive environment into immune-supportive one, and improve the anti-tumor effect of PD-L1 inhibitor [244]. Long-term bevacizumab treatment can also result in the development of drug resistance, because other redundant tumor-derived angiogenic factors, including Ang, endothelial growth factor (EGF), hepatocyte growth factor (HGF), and PDGF are up-regulated [245, 246]. In addition, Feng and Yang found bevacizumab could down-regulate the expression of α -SMA and TGF- β 1, block the effect of VEGF on HSCs to reduce liver fibrosis and protect liver function [240]. It should be noted that bevacizumab treatment could rise bleeding risk and that adequate bleeding

prophylaxis needs to be given [247]. Moreover, M. Peck-radoslavljevic team explored the efficacy of conventional transarterial chemoembolization (cTACE) combined with bevacizumab in the treatment of HCC patients, and found that there is no improvement in either radiologic tumor response or OS in HCC patients, and even though there were severe or fatal sepsis and vascular side effects. Therefore, it is concluded that bevacizumab can not be used as an adjuvant therapy for TACE [248].

Ramucirumab is a recombinant human IgG1 monoclonal antibody that interferes with high affinity with the extracellular domain of VEGFR-2, blocking the binding of its ligands VEGF-A, VEGF-C, and VEGF-D. It plays a critical role in tumor angiogenesis and tumor growth [161, 225, 249, 250]. Ramucirumab was first approved in the US in 2014 for the treatment of advanced or metastatic gastric cancer or gastro-oesophageal junction adenocarcinoma in patients [251]. In the REACH trial, a global, randomized, double-blind, placebo-controlled, phase III study of advanced HCC after first-line sorafenib, ramucirumab was tested as second-line treatment and did not significantly improve OS compared with placebo in the intention-to-treat population. However, compared with the placebo group, the OS rate in the ramucirumab group was significantly improved in the pre-defined subgroup of patients with a baseline alpha fetoprotein (AFP) of 400 ng/ml or higher [166, 252, 253]. Based on the findings of REACH trial, the randomized, double-blind phase III REACH-2 trial showed an improvement in OS in the Ramucirumab group compared with placebo in HCC patients who had previously received sorafenib and AFP concentrations of at least 400 ng/mL [254, 255]. Based on the results of REACH and REACH-2 trial, ramucirumab as second-line treatment for HCC patients with elevated $\text{AFP} \geq 400$ ng/mL pretreated with sorafenib was approved by FDA on May 10th, 2019 [256-259].

6.3 Other anti-angiogenesis drugs

Trebananib sequesters Ang-1 and Ang-2 to prevent interaction with Tie-2 receptor but Trebananib combined with Sorafenib did not further improve the survival rate of patients with advanced HCC [260].

Celecoxib, which is a selective Cyclooxygenase-2 (COX-2) inhibitor, combined with Octreotide can improve TAA induced liver fibrosis and PHT by inhibiting angiogenesis in rats [261].

Non selective beta blockers (NSBBs) may have a strong inhibitory effect on angiogenesis in cirrhosis and Carvedilol, the third generation of NSBBs, is recommended to reduce PTH [262].

Rapamycin, an immunosuppressive drug, blocked the angiogenesis of mesenteric tissue and reduced mesenteric blood flow in portal hypertensive mice, at least in part through its anti VEGF activity and its effect on the mTOR signaling pathway [263]. High dosage of Rapamycin can reduce hypoxia, decrease intrapulmonary shunt and improve Hepatopulmonary syndrome (HPS) in cirrhotic rats by down regulating mTOR / P70S6K, NF- κ B and VEGF signaling pathway [264].

6.4 Traditional Chinese medicine (TCM)

TCM has unique advantages in anti-liver fibrosis, cirrhosis and HCC due to its multi-target pharmacological action including anti-angiogenesis efficacy [265-271].

Yiguanjian Decoction (YGJ) has anti-angiogenic effects in cirrhotic mice induced by CCl₄ through inhibiting HIF-1 α /VEGF signaling pathway and improving the hepatic hypoxic microenvironment [266].

Xuefu Zhuyu decoction, Fuzheng Huayu decoction and Danggui Buxue Tang

play part in improving liver fibrosis by reducing expression of HIF-1 α , VEGF, VEGFR-2, or Ang1 and TGF- β 1, attenuate hypoxia, improving oxidative stress, and protect the function of hepatic sinusoidal endothelial cells [267-269].

Xiayuxue Decoction could inhibit angiogenesis by decreasing the activities of matrix metalloproteinase 2 (MMP-2) and MMP-9, hampering the activation of HSCs, and damaging the new vessel integrity [270].

Astragalus membranaceus and Curcuma wenyujin increased CD34 and reduced HIF-1 α , promoted vascular normalization in tumor-derived endothelial cells of HCC [271].

7. Conclusions

Considering the indisputable importance of neovascularization in the development and progression of liver fibrosis, blocking neovascularization may provide a method to reduce liver tissue distortion, improve liver fibrosis or prevent more serious damage, including cirrhosis and HCC [7, 68, 103]. As aforementioned, some achievements have been made with anti-angiogenic therapy in recent years. Targeted therapy to VEGF/VEGFR, PDGFR, or other pro-angiogenic factors have improved liver fibrosis, decreased PHT, reduced splanchnic blood flow, inhibited tumor vascularization or prolonged OS [5, 7, 158, 178-184, 218, 240, 273].

8. Expert opinion

Liver fibrosis is the common feature of almost all CLDs. Liver fibrosis is closely related to liver cirrhosis and HCC, which both often occur at the terminal stage of progressive liver fibrosis.

Pathological elements that contribute to fibrosis progression are also commonly

observed in liver cirrhosis and HCC, and many are associated with HCC development. Angiogenesis, the process of forming new blood vessels, is one of the most important pathological elements in the development and progression of any liver fibrosis, cirrhosis or HCC. Liver has a unique vascular supply, where two major venous vascular systems (portal and inferior vena cava) interact with the hepatic artery. Vessels ramify successively into more branches and capillaries until converging and forming a vascular network that covers the hepatic sinusoid. Under non-pathological conditions, angiogenesis is a highly ordered and tightly regulated process with balanced interactions between pro-angiogenic and anti-angiogenic factors. These pro-angiogenic and anti-angiogenic factors, including VEGF, PDGF, FGF, angiopoietins and tie Receptors etc., are similar among liver fibrosis, cirrhosis and HCC. During the process of liver fibrosis, persistent inflammation and hypoxia would result in hepatic sinusoidal capillarization, intrahepatic shunt, intrahepatic vascular reconstruction. In the meantime, intrahepatic resistance increased and effective perfusion of hepatocytes decreased. Due to the immaturity and permeability of VEGF induced neovascularization, pathological angiogenesis might not be able to correct liver hypoxia. Hypoxia and pathological angiogenesis may cooperate to disrupt normal tissue repair, thus promoting the development of liver fibrosis. This vicious circle between fibrosis and pathological angiogenesis can further aggravate hypoxia. When progressing from liver fibrosis to cirrhosis, the vascular structure remains irregular, but with only a few neovascularization that can anastomose with the portal vein, some of which have blind ends. Then the vessels enclosed by the fibrous tissue lead to high liver resistance and portal hypertension. The effective perfusion of hepatocytes further decreases, hypoxia aggravates, stimulates the generation of blood vessels and fibers, and so the vicious circle begins. As cirrhosis ultimately progresses

to HCC, accompanied by an increase in arterial formation, a marked abnormality, irregularity and tortuosity of blood vessels, an increase in sinusoidal capillaries, and the formation of functional collateral arteries.

Although the progression of liver fibrosis, cirrhosis and HCC is closely connected, however, not all the patients with liver fibrosis will develop into cirrhosis or HCC. Understanding how angiogenesis works during liver fibrosis, cirrhosis and HCC is crucial in improving the effectiveness of anti-angiogenesis. A series of recent studies on anti-angiogenesis therapy, multi-tyrosine kinase inhibitors including sorafenib and lenvatinib could improve liver fibrosis, reduce portal pressure, decrease visceral blood flow perfusion, and prolong overall survival.

Since liver fibrosis, cirrhosis or HCC are multi-factorial diseases, and angiogenesis is only one of the factors that influence their genesis and progression, results of current approaches to treat liver fibrosis, cirrhosis and HCC using anti-angiogenic therapies and multi-targeted TKIs are unlikely to be satisfactory.

More in-depth understanding of angiogenesis factors and the relationship between angiogenesis and other aspects of the pathogenesis and transformation may be key to enabling future progress in the treatment of patients with liver fibrosis, cirrhosis and HCC [7, 274, 275]. Multi-target therapy including anti angiogenesis may be the direction of future development.

Successful antiangiogenic treatment should avoid adverse effects caused by the inhibition of normal physiological angiogenesis processes, for instance, during the repair of damaged tissues or during the growth of blood vessels in children.

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Table 1 Role of angiogenic factors in liver fibrosis, cirrhosis and HCC involved in this paper

Angiogenic factor	Role in angiogenesis in liver fibrosis, Cirrhosis and HCC	References
VEGF/VEGFR	Produced by ECs, LSECs, damaged hepatocytes and activated HSCs	7-9, 16, 26
	increasing vascular permeability	
	triggering endothelial cell proliferation	
	regulating neo-vessel lumen diameter	
	Inducing capillarization of sinusoids	
PDGF/PDGFR	Improving migration and chemotaxis of HSCs	7, 41
	Produced by ECs, LSECs	
	Stimulating HSCs proliferation, differentiation, and migration	
	Stabilizing the neovascularization architecture	
	Promoting the maturation of neovascularization	
FGF	Inducing the activation of ECs/LSECs	7, 11, 103, 121, 122
	Increasing HSCs proliferation and recruitment	
	Stimulating integrin expression	
	Inducing capillary sinuses	
	Inducing tumor neovascularization	
PIGF	Promoting the activation and proliferation of HSCs	63, 144, 145
	Promoting hepatic macrophage recruitment and activation	
	Reducing superior mesenteric blood flow and PP	
Ang-1/Ang-2/ Tie-2	Produced by HSCs	7, 13, 26, 64-67
	Stabilizing the neovascularization architecture	
	Facilitating vessel proliferation or sprouting in the presence of VEGF	
	Promoting HSC/myofibroblast migration	
LECT2/Tie1	Inhibiting portal vein angiogenesis	33, 34
	Inducing LSECs capillarization	
$\alpha V\beta 3$	Produced by ECs	8, 146, 150
	Mediating migration and proliferation of ECs	

	Driving activation of HSCs	
Apelin	promoting proliferation and migration of ECs	74- 77
	Stimulating vascular sprouting	
	Promoting vascular remodeling	
Endoglin	Increasing the expression of VEGF	143

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Table 2 Anti-angiogenesis drugs involved in this paper

Type	Drug	Targets	Effects	Mechanisms	Adverse effects	References
TKIs	Sorafenib	VEGFR-1/2/3,	anti-HCC	Reducing HIF-1 α and	Hypertension,	174-193, 272
		PDGFR- β	anti-liver	VEGF; Inhibiting tumor	Hand-foot	
		c-Kit receptor	fibrosis	vascularization; reducing	syndrome.	
			anti-cirrhosis	PP and hyperdynamic	Myelosuppression.	
				splanchnic circulation;	Hemorrhage;	
				hindering portosystemic	Myelosuppression;	
	Lenvatinib	VEGFR 1-3,	anti-tumor	Reducing VEGF and	Hypertension,	160, 166,
		FGFR 1-4,	anti-angiogen	FGF-induced	diarrhoea;	
		PDGFR α , c-Kit	esis	angiogenesis	decreased	
		and RET			appetite;	
	Regorafenib				decreased weight	166, 207-216
		VEGFR-1/2/3;	anti-tumor	Reducing VEGF, PDGF	Hypertension,	
		Tie-2, KIT, RET,	anti-angiogen	and FGF-induced	Hand-foot skin	
		RAF1, BRAF,	esis	angiogenesis	reaction; fatigue;	
	Cabozantinib	BRAFV600E,			diarrhoea	222, 225-228
		PDGFR, FGFR				
		VEGFRs, MET,	anti-tumor	Dual blockade of	palmar-plantar	
	Axitinib	AXL, RET, KIT,	anti-angiogen	VEGFR-2 and MET	erythrodysesthesia;	229-231
		and FLT3	esis		hypertension,	
					increased aspartate	
					aminotransferase,	
					fatigue, diarrhea	

			anti-angiogen esis	angiogenesis	erythrodysesthesia; hypertension; increased aspartate aminotransferase; fatigue, diarrhea	
MAbs	Bevacizumab	VEGF	anti-HCC	Neutralizing VEGF;	Proteinuria;	240-248, 272
			anti-liver fibrosis	Blocking HSCs activation	Hemorrhage; thrombotic	
			anti-cirrhosis		microangiopathy; Fistular formation and GI perforation; Wound complications; posterior reversible encephalopathy syndrome	
	Bevacizumab+	VEGF and	anti-HCC	Inhibiting VEGF induced		242-244
	atezolizumab	PD-L1	anti-liver fibrosis	angiogenesis		
	Ramucirumab	VEGFR-2	anti-HCC	treatment for HCC patients with elevated AFP ≥ 400 ng/mL	hypertension, gastrointestinal hemorrhage; infusion-related reactions; fatigue	252-259
Other	Trebananib	Ang-1 and Ang-2		Preventing Ang-1/2 of interaction with Tie-2 receptor	Fatigue; hypertension; Diarrhea; Dyspnea; palmar-plantar erythrodysesthesia	260
	Celecoxib	COX-2 inhibitor	anti-liver fibrosis	Inhibiting angiogenesis Ameliorating PHT		261

			anti-cirrhosis		
	Carvedilol	NSBBs	anti-cirrhosis	Reducing PHT	262
	Rapamycin	VEGF, mTOR	anti-cirrhosis	blocking the angiogenesis of mesenteric tissue and reduced mesenteric blood flow	263
TCM	Yiguanjian Decoction	HIF-1 α /VEGF	anti-liver fibrosis	Inhibiting VEGF induced angiogenesis	266
	Xuefu Zhuyu decoction	HIF-1 α , VEGF, VEGFR-2, or	anti-liver fibrosis	Attenuating hypoxia, improving oxidative stress, protecting LSECs	267-269
	Fuzheng Huayu decoction	Ang1 and TGF- β 1			
	Danggui Buxue Tang				
	Xiayuxue Decoction	MMP-2, MMP-9	anti-liver fibrosis	Inhibiting the activation of HSCs; damaging the new vessel integrity	270
	Astragalus membranaceus	HIF-1 α	anti-liver fibrosis	Increasing CD34 Promoting vascular normalization of HCC	271
	Curcuma wenyujin				